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(54) Title: CONDITIONAL INHIBITION OF VEGETATIVE PROPAGATION

(57) **Abstract:** The invention relates to a method for enabling rooting or grafting of a plant which contains in its genome a gene which is placed under the regulation of an inducible promoter and which codes for a rooting-inhibiting product or a product leading to rooting inhbition, comprising of treating the plant prior to production of a cutting or graft or treating the cutting or graft after production of the cutting or graft with a substance which either discontinues the effect of the rooting-inhibiting gene; and/or results in expression of a second gene likewise present in the plant which is under the regulation of an inducible promoter and the expression product of which discontinues the effect of the rooting-inhibiting gene. The invention further relates to a transgenic plant which is provided with a mechanism for rooting inhibition and a mechanism which discontinues rooting inhibition, and to a DNA construct for effecting the discontinuation of rooting inhibition.

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CONDITIONAL INHIBITION OF VEGETATIVE PROPAGATION

The present invention relates to a method for rooting plants which are protected against undesired vegetative propagation, in addition to a DNA construct and a transgenic plant which enable conditional inhibition of vegetative propagation.

Improvement stations invest a great deal of money in making new plant varieties. This money is recovered by selling the developed plants. A major problem arises here however in that the new varieties are often propagated illegally via cuttings or via grafting. An estimated 25 to 35% of all sold carnations is thus vegetatively propagated illegally (i.e. without payment of royalties). This problem occurs not only in the case of carnations, but generally in the case of all ornamental plants with high sales such as roses and chrysanthemums, in pot plants, in vegetables which are botanically fruits such as tomato, melon, cucumber, paprika, in monocotyledonous plants such as sugarcane and in ligneous plants such as fruit trees (for instance apple, pear, cherry, orange, peach, banana, pineapple) avenue trees and trees for wood production.

The present inventors have developed a technology for inhibiting root formation conditionally (under particular conditions). This technique is described in the international application W099/06579, the content of which is incorporated herein by means of reference.

The action mechanism associated with this technology is based on the following principles. The plant growth regulator auxin is involved in the induction of roots on a shoot (adventive root formation) and in side root formation (lateral root formation). Auxin occurs in some plants in a degree sufficient to induce roots on cuttings and in the case of some other plants it has to be administered additionally via a cutting powder (or via a solution).

Antagonists for these auxin-induced processes are also present in the plant. These antagonists are signal molecules such as for instance cytokinins and ethylene. Cytokinins not only inhibit rooting but can also inhibit graft union and lateral root formation. Research has shown that a very small quantity of the cytokinins zeatin or benzylaminopurine administered for a short period of time (4 hours) is capable of completely blocking _in vitro_ root induction with auxin.

The gene that codes for a protein which can play a key role in the formation of cytokinin is the isopentenyl transferase gene (_ipt_ gene) from _Agrobacterium tumefaciens_. The conversion of 5'-adenosine monophosphate + isopentenyl pyrophosphate in the cytokinin isopentenyl adenine is catalysed by the enzyme isopentenyl transferase. This enzyme is present after transcription and translation of the _ipt_ gene. Isopentenyl adenine is converted rapidly in the plant to the very active cytokinin zeatin. Cytokinin formed via the _ipt_ gene has a paracrine effect (at the location of synthesis), so that pleiotropic effects (undesired side effects) will be limited.

Other genes with which rooting or graft union can be inhibited are genes which induce cell death at the location where and the moment at which rooting or graft union would normally speaking be desirable. Regulation of genes which control cell division (cyclins and cell cycle genes, actin genes, scarecrow genes etc.) and genes which are involved in induction, formation and development of adventive root formation can be used to inhibit root formation (for instance via inhibition of functional gene expression). In addition, genes which play a part in auxin transport, such as the PIN genes, can be used to inhibit root formation and graft union.

In addition to blocking adventive root formation, the above stated genes can also be used to stop root formation at the moment when the root primordium is

being formed or has already been formed. Use is made herein of a gene promoter which is active in these stages. A properly functioning root system does not then result. Inhibition of lateral root formation with the above-stated genes and with genes which are involved in induction and formation of lateral roots can also be used to prevent the formation of a good root system (these genes must then be controlled by a promoter which is specific for the stage of lateral root formation).

An example of a gene involved in the biosynthesis of the auxin-antagonist cytokinin is the ipt gene. It is of great importance for plants propagated by means of cutting or grafting that the production of cytokinin takes place at the location where normally speaking root formation or graft union is desired, but where this must now be prevented. This can be achieved for instance with a wound-induced promoter. Because cytokinins formed after expression of the ipt gene (such as isopentenyl adenine and zeatin) are poorly transported in the plant, pleiotropic effects are limited. Expression of the ipt gene in a wounded leaf also has few adverse effects, because cytokinin is naturally involved in the recovery from wounds.

The above described system is in principle very useful in preventing undesired vegetative propagation of plants. However, it also prevents propagation when this is desired, such as by the developer or breeder of the plant itself or with the permission of the developer or breeder.

It is therefore the object of the present invention to provide a method whereby, if so required or desired, the inhibition of the vegetative propagation can be discontinued.

This is achieve with the invention by a method for enabling rooting or grafting of a plant which contains in its genome a gene which is placed under the regulation of an inducible promoter and which codes for

a rooting-inhibiting product or a product leading to rooting inhibition, comprising of treating the plant prior to production of a cutting or graft or treating the cutting or graft after production of the cutting or graft with a stimulus which:

a) discontinues the effect of the rooting-inhibiting gene; and/or

b) results in expression of a second gene likewise present in the plant which is under the regulation of an inducible promoter and the expression product of which discontinues the effect of the rooting-inhibiting gene.

Thus is ensured that the rooting inhibition is switched off. The stimulus, usually a substance which causes rooting inhibition to be discontinued directly or indirectly (via induction of the second gene), can be made available by the owner of the plant variety to those who are authorized for vegetative propagation of a plant which is patented or protected by plant breeders' rights and provided with the rooting-inhibiting mechanism.

The stimulus which discontinues the effect of the rooting-inhibiting gene can be chosen from substances which inhibit the biosynthesis of the rooting-inhibiting product and structural analogs of the rooting-inhibiting product.

Particularly suitable rooting-inhibiting substances are the endogenous plant hormones cytokinins. These can be produced in a plant in which the rooting-inhibiting mechanism is active via expression of the <u>ipt</u> gene, the product of which plays a key part in the cytokinin biosynthesis.

The stimulus, in particular the substance, which inhibits the biosynthesis of the cytokinin is a substance which is a structural analog of a precursor of a cytokinin, such as the substrates of IPT from which

isopentenyl adenine and subsequently, via other enzymes, zeatin is made. Examples are shown in Table 1A.

When inhibition is effected via a competition mechanism, structural analogs of the cytokinin can be applied. Suitable examples hereof are shown in Table 1B.

Table 1A

Structural analogs of precursors

<u>Analogs of 5-adenosine monophosphate:</u>

8-aza-adenosine
1,7 or 9)-deaza-adenosine
8-bromo-3-ribosyl
6-phenylamino-deoxyadenosine
7-deaza-adenosine (tubercidin)
8-bromo-adenosine
8-bromo-adenosine 5'-monophosphate
3-deaza-adenosine

<u>Analogs for IPP:</u>

aminophenethyl pyrophosphate (APP).
dimethylallyl pyrophosphate

Table 1B

Anti-cytokinins (structural analogs of cytokinins)

N-(4-pyridyl)-O-(4-chlorophenyl)-carbamate (PC)
4-cyclopentylamino-2-methylthio-pyrrolo[2,3-d]pyrimidine (CCP)
various 4-substituted 2-methylpyrrolo[2,3-d]pyrimidines, including:
4-cyclobutylamino-2-methylpyrrolo[2,3-d]pyrimidine (CBP)
4-cyclobutylamino-2-methylpyrrolo[2,3-d]pyrimidine (CBP)
4-cyclopentylamino-2-methylpyrrolo[2,3-d]pyrimidine (CPP),
3-methyl-7-(pentylamino)pyrazolo(4,3-d)pyrimidine,
3-methyl-7-(benzylamino)pyrazolo(4,3-d)pyrimidine,
7-(n-pentylamino)-3-(β-D-ribofuranosyl)pyrazolzolo [4,3-d]pyrimidine,

7-cyclopentylamino-3-(β-D-ribofuranosyl)pyrazolzolo [4,3-d]pyrimidine,
7-n-hexylamino-3-(β-D-ribofuranosyl)pyrazolzolo[4,3-d]pyrimidine,
4-(3-methyl-2-butenylamin)pyrazolo[3,4-d]pyrimidine-3-carboxamide
4-(3-methylbutenylamin)pyrazolo[3,4-d]pyrimidine-3-carboxamide
4-substituted triazolo[4,5,d]pyrimidines
4-substituted pyrazolo (3,4-d)pyrimidines
4-substituted triazola(4,5,d)pyrimidines
4-cyclopentylamino-2-methylthiopyrrolo(2,3-d)pyrimidine
2-cyclobutylamino-4-ethylamino-6-chloro-1,3,5-triazine
alkyl and phenyl N-phenyl carbamates
4-chloro-2-cyclobutylamino-6-ethylamino-s-triazine
N-6(phenyl)adenine
1-phenyl-3-(8-quinolinyl)urea
1-(4-methoxyphenyl)-3-(8-quinolinyl)urea
1-phenyl-3-((2,6-tetramethyl-)4-piperinyl))urea
1-phenyl-3-((2,2-6,6-tetramethylpiperidine)-4)urea
1-phenyl-3-((2-tritylethaneamine)-1)urea
1-phenyl-3-(2-aminoethane)urea-hydrochloride
1-phenyl-3-((1,3,4-thiadiazole)-2)urea
1-phenyl-3-((1,3-thiazole)-2)urea
1-(4-methoxyphenyl)-3-((2,2-6,6-tetramethylpiperidine)-4)urea
1-phenyl-3-(leucyl)urea
1-phenyl-3-(8-octanoic)urea
1-phenyl-3-(pyrazyl)urea
1-phenyl-3-(2-pyrimidyl)urea
nicotinic acid-N-(leucyl-amide)
((4-methyl)-1-succinic acid)-N-(4-pyridyl)-amide
1-(4-methoxyphenyl)-3-(4-pyridyl)urea
benzylaminopurine-N9-propionic acid
1-phenyl-3-(glycyl)-urea

An alternative mechanism for discontinuing rooting inhibition and inhibition of graft union takes place via a genetic route. For this purpose the plant must contain a second gene in its genome. As soon as it is expressed, this gene results in the discontinuation of the rooting inhibition. This can take place in different ways.

The second gene can thus be a gene coding for the antisense version of the rooting-inhibiting gene. The messenger transcribed from the antisense version of the gene captures the mRNA of the sense version whereby no translation to protein takes place. The protein is the rooting inhibitor itself or is required in the biosynthesis thereof. Now the product is no longer formed, the rooting inhibition is discontinued.

The second gene preferably codes for an antisense version of the ipt gene.

The second gene can further be a gene which codes for a second sense version of the rooting-inhibiting gene. Co-suppression can hereby be effected. The second gene preferably codes for a sense version of the ipt gene.

The second gene can also be one of the rolABC genes of Agrobacterium rhizogenes. Expression of the rolABC genes makes the plant more insensitive to cytokinin.

In an alternative embodiment the first gene is under the regulation of an inducible promoter which is modified, for instance a wound-inducible promoter which regulates expression of the ipt gene. The modification ensures that a repressor can interact with the promoter. The second gene, the expression of which is likewise inducible (for instance via a chemical substance or via another method), codes for a repressor-protein which inhibits expression of the first gene. An example hereof is the GAL4 repressor system. The rooting-inhibiting gene is therefore preferably the ipt gene, which is under the regulation of for instance a modified wound-inducible promoter, wherein the modification is such

that the repressor of the GAL4 regulation system can interact therewith.

The second gene can further code for a degradation enzyme of the rooting-inhibiting gene product. The rooting-inhibiting gene product is preferably cytokinin and the second gene then codes for instance for cytokinin-oxidase.

Finally, the second gene can be a mutated cytokinin receptor gene, whereby the cytokinin pathway cannot take place and the rooting becomes conditional.

In all these cases the expression of the second gene is inducible (for instance with a chemical compound) and can therefore be switched on at the moment rooting is required by administering the inducer. Induction proceeds via the promoter. Examples hereof are gene-regulation systems which make use of promoters which are inducible with for instance ethanol, copper ions (or other metals), tetracycline, dexamenthasone (and other glucocorticoid promoters), benzothiadiazole, N-(aminocarbonyl)-2-chlorobenzene sulfonamide or tebufenozide.

The invention further relates to a transgenic plant which is reversibly protected against undesired vegetative propagation, which plant contains in its genome a first gene which is placed under the regulation of an inducible first promoter and which codes for a rooting-inhibiting product or a product leading to rooting inhibition, and a second gene which is placed under the regulation of a second promoter inducible by means of another stimulus and which codes for a product which deactivates the rooting-inhibiting expression product of the first gene.

The invention further relates to a DNA construct comprising transcription-initiation sequences, including an inducible promoter, and optionally transcription termination sequences operably linked to a second gene as defined above.

The same DNA construct can advantageously further contain a first gene which codes for a rooting-inhibiting product or a product leading to rooting inhibition provided with transcription-initiation sequences operably linked thereto, including an inducible promoter, and optionally transcription termination sequences. The first gene is advantageously the ipt gene.

The second gene can in both the transgenic plant and the DNA construct be everything as defined above.

The invention further relates to a method for reversing rooting inhibition in a cutting or the growing together of a rootstock and upper stem (graft union) of a transgenic plant as described above, comprising of treating the cutting or graft with the stimulus of the second promoter and placing the cutting or graft on a rooting medium.

According to the invention a stimulus, usually a substance, must therefore always be administered which ensures that the mechanism whereby the inhibition of the rooting is discontinued is set into operation. In the case of a substance, this advantageously forms part of a composition, such as for instance a cutting powder or a graft promoter (substance which enhances the growing together of the graft with the lower stem) or a solution. Rooting or graft union is then only possible in the presence of this cutting powder, the graft promoter or a solution thereof. The cutting powder or graft promoter (or solutions thereof) can be administered after making the cutting or graft. Conversely, the plants from which the graft or cutting is made can also be treated beforehand with the substance for inhibiting the effect of the ipt gene.

Examples of the substances which discontinue the rooting inhibition directly, i.e. not via the genetic route, are mentioned above. In addition to these substances, other cytokinin inhibitors can be readily

found among the arsenal of available substances. Finding these other substances can be done by testing a large number of substances in so-called high throughput screening (HTS) systems. Many thousands of compounds can be tested in the HTS systems. The IPT protein (or bacteria containing or producing the IPT-protein) is combined with cytokinin precursors (5'-adenosine monophosphate and isopentenylpyrophosphate) and potential inhibitors for testing. It is then determined, with for instance gas chromatograph-mass spectrograph (GC-MS) or with antibodies in an ELISA or RIA test, whether isopentenyl adenine (IP) or zeatin is formed. The effectiveness of the substances which inhibit IP or zeatin formation van be further optimized via so-called "combinatorial chemistry". All this may result in the identification of substances which are suitable for direct application to discontinue the ipt activity in the plant (conditional inhibition of rooting).

The action of the genetic mechanism whereby rooting inhibition is discontinued is based on expression induction. The second gene which discontinues the activity of the rooting-inhibiting product is switched on at the desired moment by induction via its promoter. Inducible promoters are for instance the per se known systems inducible using alcohol and tetracycline. Other examples of inducers are copper ions (or other metals), dexamenthasone (and other glucocorticoid promoters) benzothiadiazole, N-(aminocarbonyl)-2-chlorobenzene sulfonamide or tebufenozide.

It is further possible to provide the DNA constructs containing the rooting-inhibiting gene and/or the gene which leads to discontinuation of the rooting inhibition with a marker gene, such as GUS or GFP. It is then possible throughout the whole chain - from producer tot consumer - to perform a very rapid test for the presence of the reporter, such as by means of a GUS assay or using a fluorescence meter. The construct which

is incorporated can be identified via a Southern blot or PCR reaction.

In the present application "rooting-inhibiting gene" is understood to mean a gene which codes for a rooting-inhibiting product or product leading to rooting inhibition, or a gene which codes for a product which can prevent graft union or which results in prevention of graft union. The gene product of the rooting-inhibiting gene is referred to as "rooting-inhibiting gene product". Dit product is itself not necessarily rooting-inhibiting, but in any case results in inhibition of rooting or prevention of graft union.

The present invention will be further illustrated on the basis of the example following below which is only given by way of elucidation and should not be deemed as limiting.

EXAMPLE

Conditional root formation by applying anti-cytokinin on plant tissues which express the ipt-gene

Examined in this example is the *in vitro* effect of anti-cytokinin on auxin-induced root induction on transgenic tobacco plants (cultivar SR1), which carry in them the *ipt* gene downstream of the *rolB* promoter.

First was demonstrated that the *rolB* promoter is switched on by auxin. This was found in experiments with groups of tobacco plants (cultivar SR1, obtainable in the regular seed trade) which were transformed with different gene constructs. These plants were transformed with the *rolB* promoter which regulated either the glucuronidase (GUS) gene or the rolB gene (i.e. the *rolB* promoter is located upstream of the rolB gene). Stem slices of the *rolB*-rolB and *rolB*-GUS shoots were incubated *in vitro* on rooting medium without auxin or on rooting medium with different concentrations of the auxins indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA). The *rolB*-GUS shoots displayed an increase in

GUS-expression in the GUS-assay after incubation for different periods on auxin-containing medium (in comparison with shoots incubated on medium without auxin).

In accordance herewith it was demonstrated on a Northern blot, which was made of rolB-rolB stem slices of tobacco, that auxin induced the expression of the rolB gene. Finally, expression of the ipt gene resulted in rolB-ipt SR1 tobacco tissue in a very strong increase in the formation of axillary shoots (between ten and twenty relative to the control plants which formed no axillary shoots), which points to an increased cytokinin content via expression of the ipt gene.

Twenty of these transgenic rolB-ipt tobacco shoots were incubated for three weeks on Murashige and Skoog medium with 2% sucrose and the auxins indol-3-butyric acid (IBA) or naphthalene acetic acid (NAA). The auxins were present during the entire incubation period in the following concentrations: 0, 1, 3.2, 10 and 100 µM. As control the same incubations were performed with wild type SR1 tobacco shoots. The transgenic tobacco plants formed no roots. The wild type control shoots formed roots at all auxin concentrations. Strong callus formation was observed at 100 µM auxin.

The rolB-ipt SR1 tobacco shoots were incubated with 10 µM IBA plus 10 or 100 µM of the anti-cytokinin 2-cyclobutylamino-4-ethylamino-6-chloro-1,3,5-triazine. These shoots (see figure) were able to form roots, in contrast to the shoots which were incubated without anti-cytokinin. This shows that the effect of expression of the ipt gene can be prevented by anti-cytokinins. Other anti-cytokinins are listed in Table 1B.

Anti-cytokinin activity can also be obtained via introduction of a gene construct which prevents the effect of expression of the ipt gene, as explained in the description introduction.

The techniques used for the above stated experiments are described in the following references:

1. Cutting stem slices, incubation methods and in vitro techniques: Van der Krieken WM, Breteler H, Visser MHM, Mavridou D. 1993. The role of the conversion of IBA into IAA on root regeneration in apple: introduction of a test system. Plant Cell Reports 12, 203-206.
2. The rooting medium: Quoirin M, Lepoivre P, Boxus P. 1977. Un premier bilan de 10 années de recherches sur les cultures de méristemès et la multiplication in vitro de fruitiers ligneux. In Comptes Rendus de Recherches 1976-1977 de la Station des Cultures Fruitieres et Maraicheres. Gembloux, Belgium, 93-117.
3. The MS medium: Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473-497.

CLAIMS

1. Method for enabling rooting or grafting of a plant which contains in its genome a gene which is placed under the regulation of an inducible promoter and which codes for a rooting-inhibiting product or a product leading to rooting inhibition, comprising of treating the plant prior to production of a cutting or graft or treating the cutting or graft after production of the cutting or graft with a substance which:

a) discontinues the effect of the rooting-inhibiting gene; and/or

b) results in expression of a second gene likewise present in the plant which is under the regulation of an inducible promoter and the expression product of which discontinues the effect of the rooting-inhibiting gene.

2. Method as claimed in claim 1, **characterized in that** the substance which discontinues the effect of the rooting-inhibiting gene is chosen from the group consisting of substances which inhibit the biosynthesis of the rooting-inhibiting product and structural analogs of the rooting-inhibiting product.

3. Method as claimed in claim 2, **characterized in that** the rooting-inhibiting product is a cytokinin.

4. Method as claimed in claim 3, **characterized in that** the substance which inhibits the biosynthesis of the cytokinin is chosen from the group consisting of substances from Table 1A.

5. Method as claimed in claim 3, **characterized in that** the structural analog of cytokinin is chosen from the group consisting of substances from Table 1B.

6. Method as claimed in claim 1, **characterized in that** the second gene is a gene coding for the antisense version of the rooting-inhibiting gene.

7. Method as claimed in claim 6, **characterized in that** the second gene codes for an antisense version of the ipt gene.

8. Method as claimed in claim 1, **characterized in that** the second gene is a gene which codes for a second sense version of the rooting-inhibiting gene.

9. Method as claimed in claim 8, **characterized in that** the second gene codes for a sense version of the ipt gene.

10. Method as claimed in claim 1, **characterized in that** the second gene is one or more of the rolABC genes of Agrobacterium rhizogenes.

11. Method as claimed in claim 1, **characterized in that** the second gene codes for a repressor of the promoter of the rooting-inhibiting gene.

12. Method as claimed in claim 11, **characterized in that** the rooting-inhibiting gene is the ipt gene and is under the regulation of a modified wound-inducible promoter, wherein the modification is such that the repressor of the GAL4 regulation system can interact therewith.

13. Method as claimed in claim 1, **characterized in that** the second gene codes for a degradation enzyme of the rooting-inhibiting gene product.

14. Method as claimed in claim 13, **characterized in that** the rooting-inhibiting gene product is cytokinin and the second gene codes for cytokinin-oxidase.

15. Method as claimed in claim 1, **characterized in that** the second gene codes for a mutated receptor for the rooting-inhibiting gene product in order to block the signal transduction pathway of the rooting-inhibiting gene product.

16. Method as claimed in claim 15, **characterized in that** the rooting-inhibiting gene product is cytokinin and the second gene codes for a mutated cytokinin receptor.

17. Transgenic plant which is reversibly protected against undesired vegetative propagation, which plant contains in its genome a first gene which is placed under the regulation of an inducible first promoter and which codes for a rooting-inhibiting product or a product leading to rooting inhibition, and a second gene which is placed under the regulation of a second promoter inducible by means of another stimulus and which codes for a product which deactivates the rooting-inhibiting expression product of the first gene.

18. Transgenic plant as claimed in claim 17, **characterized in that** the second gene is a gene which codes for the antisense version of the rooting-inhibiting gene.

19. Transgenic plant as claimed in claim 18, **characterized in that** the second gene codes for an antisense version of the ipt gene.

20. Transgenic plant as claimed in claim 17, **characterized in that** the second gene is a gene which codes for a second sense version of the rooting-inhibiting gene.

21. Transgenic plant as claimed in claim 20, **characterized in that** the second gene codes for a sense version of the ipt gene.

22. Transgenic plant as claimed in claim 17, **characterized in that** the second gene is one or more of the rolABC genes of Agrobacterium rhizogenes.

23. Transgenic plant as claimed in claim 17, **characterized in that** the second gene codes for a repressor of the promoter of the rooting-inhibiting gene.

24. Transgenic plant as claimed in claim 23, **characterized in that** the rooting-inhibiting gene is the ipt gene and is under the regulation of a modified wound-inducible promoter, wherein the modification is such that the repressor of the GAL4 regulation system can interact therewith.

25. Transgenic plant as claimed in claim 17, **characterized in that** the second gene codes for a degradation enzyme of the rooting-inhibiting gene product.

26. Transgenic plant as claimed in claim 25, **characterized in that** the rooting-inhibiting gene product is cytokinin and the second gene codes for cytokinin-oxidase.

27. Transgenic plant as claimed in claim 17, **characterized in that** the second gene codes for a mutated receptor for the rooting-inhibiting gene product in order to block the signal transduction pathway of the rooting-inhibiting gene product.

28. Transgenic plant as claimed in claim 27, **characterized in that** the rooting-inhibiting gene product is cytokinin and the second gene codes for a mutated cytokinin receptor.

29. Method for reversing rooting inhibition in a cutting or graft of a transgenic plant as claimed in claims 17-28, comprising of treating the cutting or graft with the stimulus of the second promoter and placing the cutting or graft on a rooting medium.

30. DNA construct comprising transcription-initiation sequences, including an inducible promoter, and optionally transcription termination sequences operably linked to a second gene as defined in the characterizing part of claims 6-16.

31. DNA construct as claimed in claim 30, further comprising a first gene which codes for a rooting-inhibiting product or a product leading to rooting inhibition provided with transcription-initiation sequences operably linked thereto, including an inducible promoter, and optionally transcription termination sequences.

32. DNA construct as claimed in claim 31, **characterized in that** the first gene is the ipt gene.

Rooting of *rolB*-ipt tobacco with 10 μM IBA plus anti-cytokinin